ANTIBIOFILM PERFORMANCE OF SILVER NANOPARTICLES AND NEW SILVER NANOPARTICLE WOUND DRESSING

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BACKGROUND

Structured aggregates of bacteria, which can survive hostile environmental conditions as well as exhibit resistance to the host's immunity and different chemotherapeutic agents are called biofilms. They comprise of single or multiple species of bacteria enclosed in an extracellular polymeric substance (EPS) that is mainly composed of polysaccharides, nucleic acids, and proteins. Since infections caused by biofilm-forming bacteria are difficult to treat, there is a need to search for novel substances which would prevent biofilm formation as well as eradicate already mature biofilms.

The methods in this study have been chosen based on being reproducible methods, that provide in vitro data to establish if there is a potential for silver nanoparticle dressing to disrupt the biofilm. Many models are available with increasing complexity, however the starting point was a relatively cost effective test method. S. aureus and P. aeruginosa were the first bacteria to be tested because of their prevalence and because they are the bacteria most widely tested in biofilm models.

METHODS

Biofilm prevention – Silver nanoparticle solution

Biofilm prevention testing has been performed as described in literature[1]. For quantitative estimation of biofilm formation, the microtiter plate assay was used. In this method, 96-well microtiter plates were used. Using BHI (brain heart infusion) broth (instead of Muller Hinton as BHI promotes biofilm), silver nanoparticles (AgNPs) were double diluted from 50%-0.0001% and inoculated. After incubation at 37°C for 24 hours, the content of the wells was removed gently and washed thrice with Phosphate-buffered saline (PBS) to remove any planktonic bacterial cells. Remaining bacteria/ biofilms were fixed with sodium acetate followed by staining with crystal violet dye (0.1%) for 10 minutes. The stained cells attached to wells were then washed with distilled water and dried. 200 µl of 95% ethanol was added in each well to elute the attached cells, and absorbance was measured at 620 nm on ELISA reader in order to quantify cells capable of forming biofilms. Negative and positive controls were also used in the assay using sterile growth medium only and working solution, respectively. Two strains of bacteria have been tested: P.aeruginosa and MRSA, each tested in triplicate.

Biofilm eradication - Silver nanoparticle solution

Biofilm eradication 48 hours testing has been performed following literature[2]. Instead of using peg method all biofilms were formed in 96 well plates. This assay can be read by sight (looking for turbidity in wells) and by a minimum bactericidal concentration (MBC) type method where 10µl is removed and placed on agar plate and incubated to calculate.
AgNP concentration range was the same as in biofilm prevention testing: 50%-0.0001% of neat concentration.
Two strains of bacteria have been tested: P.aeruginosa and MRSA, each tested in triplicate.

Glass plate model - Silver nanoparticle dressing

Glass plates with biofilm were transferred to a humid chamber and supplied with 4ml 0.9% NaCl solution. Before covering of the glass plates the test dressing was pre-wetted with 12ml 0.9%NaCl, while cotton gauze pads were used directly. Plates left untreated (w/o dressing) were included as control for biofilm growth. All plates with or without dressings were incubated for 24h at 37°C. Afterwards, biomass was quantified by determination of the bacterial respiratory activity with the use of a fluorescence method.

Mucoid Biofilm model – Silver nanoparticle dressing

Test dressing samples were aseptically prepared to 5 cm x 5 cm sections and activated using 1.5 ml of Phosphate-buffered saline (PBS) solution. Following 7 days incubation, test dressing samples were placed on top of the porcine skin samples with biofilm and incubated aerobically for 24 hours at 37°C±2°C in a humidified environment.

RESULTS

Silver nanoparticle solution

Table 1. Silver nanoparticle concentration needed for biofilm eradication and prevention.

Bacteria	Repeat	Biofilm eradication (ppm)	Biofilm prevention (ppm)
P. aeruginosa	1	1.7	0.1
	2	1.7	0.1
	3	0.9	0.1
Average	-	1.4	0.1
MRSA	1	6.9	3.4
	2	3.4	3.4
	3	3.4	3.4
Average	-	4.6	3.4

Results suggest that the AgNPs in solution are very effective in preventing biofilms even at sub-MIC levels for both gram positive and gram-negative bacteria. The AgNPs are more effective on gram-negative than gram-positive, which is the predicted trend. It is well-documented in literature that this trend is normal, taking into consideration that MRSA can be quite resistant to silver, especially environmental MRSA strains as gram positives have historically had more exposure than gram negatives.

Results for biofilm eradication show the same trend as for biofilm prevention, meaning that silver nanoparticles are more effective against gram negative bacteria, although they are still very effective against gram positive bacteria as well.

Glass plate model – Silver nanoparticle dressing

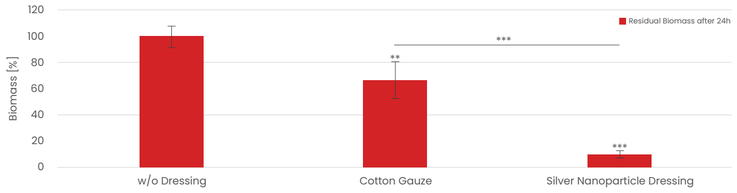

Figure 1: Effect of silver nanoparticle dressing and cotton gauze on Staphylococcus aureus biofilm biomass designated as [%] compared to the control w/o dressing.

Coverage of glass plates with a S. aureus biofilm for 24 hours with cotton gauze led to a distinct reduction of biomass upon removal of the dressing. The silver nanoparticle wound dressing exhibited a higher capacity to reduce biomass compared to the control without dressing as well as cotton gauze. In accordance, no viable bacteria were observed in the silver nanoparticle dressing, while the bacteria removed by cotton gauze were still viable in the dressing (Figure 2).

Figure 2: Evaluation of the viability of Staphylococcus aureus biofilm bound by silver nanoparticle dressing and cotton gauze designated as [%] compared to the control w/o dressing.

Mucoid biofilm model – Silver nanoparticle dressing

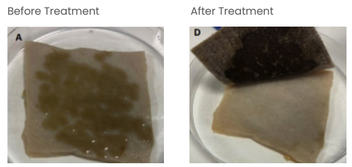

Figure 3: Perfectus Biomed Mucoid Model before and After Images

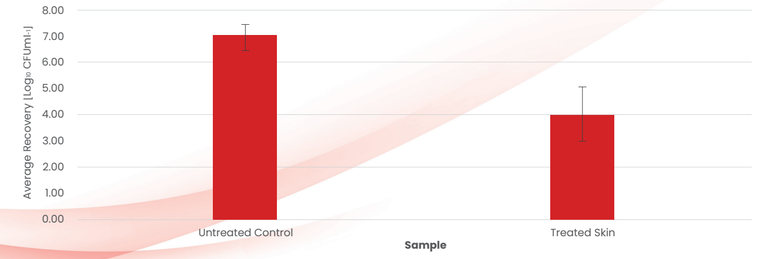

Figure 4: Average recovery for P. aeruginosa obtained from the skin biopsies after treatment.

Following 7 days incubation with Pseudomonas aeruginosa, an average Log recovery of 6.99 was observed for the 8 mm untreated skin biopsy and 3.95 was observed for the 8 mm treated skin biopsy. This was an average Log reduction of 3.04 ($p < 0.05$) following 24 hours treatment with test dressing samples, compared to the untreated skin samples at 7 days (Figure 4).

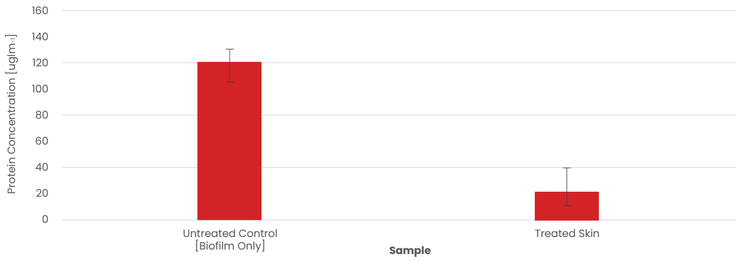

Figure 5: Average total protein content on pig skin following 7 days incubation.

Following 7 days incubation with P. aeruginosa, an average of 118.39 µg/mL in total protein was measured from the 8 mm untreated skin biopsy and 23.76 µg/mL in total protein from treated skin biopsy. This was an average reduction of 94.63 µg/mL (79.93 %) in total protein following 24 hours treatment with test dressing samples, compared to the untreated skin samples at 7 days (Figure 5).

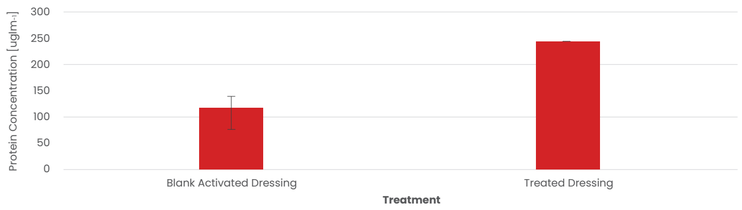

Figure 6: Average total protein content in the dressing following 7 days incubation.

Following 24 hours treatment using the test dressing, an average of >243.69 µg/mL was obtained from the test dressing samples. An average of 109.31 µg/mL was recovered from the blank activated samples. This was an average increase of >134.38 ± 0.00 ug/ml in total protein for treated dressing samples, compared to the blank activated dressing samples (Figure 6).

Conclusions

- Silver nanoparticle solution is very effective when it comes to prevention and eradication of Biofilms produced by gram positive and gram-negative bacteria. Results show that silver nanoparticles are slightly more effective in prevention and eradication of gram-negative bacteria biofilms (P.aeruginosa) with required concentrations: 0.1 ppm and 1.4 ppm, respectively. To prevent and eradicate MRSA biofilm higher AgNP concentration is needed: 3.4 ppm and 4.6 ppm, respectively.
- Glass plate model results show that the application of the silver nanoparticle wound dressing significantly reduced biofilm biomass compared to a control without dressing and the use of cotton gauze. Moreover, bacteria were rapidly killed under the test conditions.
- A significant 80% reduction in total protein was observed between the untreated and treated 8 mm biopsy punch skin samples, whereas a significant > 105.83% increase was observed between the total protein within the blank activated and treated dressing samples. This correlation of a reduction in viable bacterial count data and total protein from 8 mm biopsy punch samples, alongside the increase in total protein observed within the dressing samples following treatment, greatly increases the confidence in silver nanoparticle wound dressing ability to help control the mucoid wound exudate associated with a chronic wound.

REFERENCES

1. Muhammad Hussnain Siddique, Bilal Aslam, Muhammad Imran, Asma Ashraf, Habibullah Nadeem, Sumreen Hayat, Mohsin Khurshid, Muhammad Afzal, Imran Riaz Malik, Mudassar Shahzad, Umber Qureshi, Zia Ul Haq Khan, Saima Muzammil, 'Effect of Silver Nanoparticles on Biofilm Formation and EPS Production of Multidrug-Resistant Klebsiella pneumoniae', BioMed Research International, vol. 2020, Article ID 6398185, 9 pages, 2020. https://doi.org/10.1155/2020/6398185
2. Moskowitz SM, Foster JM, Emerson J, Burns JL. 'Clinically feasible biofilm susceptibility assay for isolates of Pseudomonas aeruginosa from patients with cystic fibrosis.' J Clin Microbiol. 2004 May;42(5):1915-22. doi: 10.1128/JCM.42.5.1915-1922.2004. PMID: 15131149; PMCID: PMC404629.